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Phylogenetic relationships in *Solanum* (Solanaceae) based on *ndhF* sequences

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ABSTRACT. A phylogenetic analysis was conducted using sequence data from the chloroplast gene *ndhF*. Sequences were obtained from 25 species of Solanaceae, including 18 species of *Solanum* representing five of the seven conventionally recognized subgenera. Trees were constructed using parsimony and maximum likelihood methods. Results indicate that *Solanum lycopersicum* (formerly in genus *Lycopersicon*) and *Solanum betaceum* (formerly in genus *Cyphomandra*) are nested within the *Solanum* clade. Each of the *Solanum* subgenera *Leptostemonum*, *Minon*, *Potatoe*, and *Solanum* are not monophyletic as currently circumscribed. Four major clades within *Solanum* are supported by high bootstrap values, but the relationships among them are largely unresolved. The problematical sections *Aculeigerum* (represented by *S. wendlandii*) and *Allophyllum* (represented by *S. allophyllum*) emerge as sister taxa in a larger clade composed of *S. betaceum*, *S. luteoalbum*, and members of subgenera *Leptostemonum*, *Minon*, and *Solanum*. Several prominent morphological characters such as spines, stellate hairs, and tapered anthers apparently have evolved more than once in *Solanum*.

Solanum L. is one of the largest and most economically important genera of angiosperms. Although the precise number of species included in *Solanum* is still unclear, estimates range from about 1,000 to nearly 2,000 species (Correll 1962; Seithe 1962; D'Arcy 1979, 1991; Nee 1993). Major crop species included in *Solanum* in the traditional sense include the potato (*S. tuberosum* L.) and eggplant (*S. melongena* L.) as well as species of lesser importance as sources of food and medicinal or poisonous alkaloids. Other economically important genera such as the tomatoes (*Lycopersicon* Mill.) and tree tomatoes (*Cyphomandra* Mart. ex Sendtn.) have been considered to be closely related to *Solanum*. Recent phylogenetic studies (Olmstead and Palmer 1992, 1997; Spooner et al. 1993; Bohs and Olmstead, in press; Olmstead et al., in press) confirm the derivation of these two genera from within *Solanum*.

Traditional classifications recognize two subfamilies, the Solanoideae and the Cestroideae. *Solanum* is the largest genus in the Solanoideae, whose members are characterized by flattened seeds with curved embryos (Hunziker 1979). Within the Solanoideae, *Solanum* has been placed traditionally in the large and complex tribe Solaneae. Evolutionary relationships among the approximately 34 genera of the Solaneae still are understood imperfectly, but recent molecular systematic work by Olmstead and Palmer (1992, 1997), Bohs and Olmstead (in press), and Olmstead et al. (in press) indicates that *Solanum* may be most closely related to genera such as *Capsicum* L., *Jaltomata* Schltdl., and *Lycianthes* (Dunal) Hassl. *Solanum* itself has been set apart from other genera in the Solaneae by having poricidally dehiscent anthers and lacking specialized calyx teeth (as in *Lycianthes*), anther beaks (as in *Lycopersicon*), or enlarged anther connectives (as in *Cyphomandra*). However, subfamilial, tribal, and generic circumscriptions in the Solanoideae remain in a state of flux.

According to the widely used scheme of D'Arcy (1972, 1991), *Solanum* is divided into seven subgenera and some 60 to 70 sections. Well-defined and probably monophyletic subgenera and sections exist along with a plethora of poorly circumscribed groups. Significant numbers of *Solanum* species have no conclusive subgeneric or sectional affiliation. Even where well-characterized infrageneric groups exist, their phylogenetic relationships to other groups generally are unknown.

Most of the taxonomic confusion surrounding *Solanum* is due to its large size, morphological variation, and predominantly tropical distribution. The last taxonomic monograph of the entire genus is over a hundred years old (Dunal 1852). Since that time, the increasing size and complexity of *Solanum* have defied a comprehensive, unified treatment.

Instead, taxonomists have examined subgroups within the genus or have treated geographically circumscribed groups of species in regional floras. Phylogenetic studies of *Solanum* subgroups or of the genus as a whole have been sparse. Cladistic analyses based on morphological characters exist for *Solanum* section *Androceras* (Nutt.) Marzell (Whalen 1979), section *Lasiocarpa* (Dunal) D'Arcy (Whalen et al. 1981; Whalen and Caruso 1983; Bruneau et al. 1995), the *S. nitidum* Ruiz & Pav. group [section *Holophylla* (G. Don) Walp. pro parte; Knapp 1989], the *S. sessile* Ruiz & Pav. group [section *Geminata* (G. Don) Walp. pro parte; Knapp 1991], subgenus *Leptostemonum* (Dunal) Bitter (Whalen 1984), subgenus *Potatoe* (G. Don) D'Arcy (Spooner et al. 1993), and subgenus *Archaesolanum* Marzell (Symon 1994). Recently, molecular phylogenetic studies have elucidated systematic problems in *Solanum* and the evolutionary placement of *Solanum* within the larger Solanoideae (Palmer and Zamir 1982; Hosaka et al. 1984; Debener et al. 1990; Spooner et al. 1991; Olmstead and Palmer 1991, 1992, 1997; Spooner and Sytsma 1992; Spooner et al. 1993; Olmstead and Sweere 1994; Bruneau et al. 1995; Bohs and Olmstead, in press; Olmstead et al., in press). Nonetheless, and many questions remain.

The present study addresses some of the systematic problems within *Solanum* and related genera using sequence data from the chloroplast gene *ndhF*. The *ndhF* region is approximately 2220 base pairs in length and codes for a subunit of a putative NADH dehydrogenase involved in chloroplast respiration (Suguira 1989, 1992). Previous studies have demonstrated the utility of *ndhF* sequence data in inferring phylogenetic relationships at the inter- and infrafamilial levels in various plant groups (Olmstead and Sweere 1994; Clark et al. 1995; Kim and Jansen 1995; Olmstead and Reeves 1995; Scotland et al. 1995; Neyland and Urbatsch 1996), due in large part to its elevated rate of base substitution compared with the chloroplast gene *rbcL* (Olmstead and Palmer 1994). The immediate goal was to determine the utility of *ndhF* sequence data in reconstructing phylogenetic relationships within *Solanum* and its relatives, and to construct a phylogeny for subgroups within *Solanum*. The resulting trees will be used to identify sister group relationships and to guide phylogenetic studies at lower taxonomic levels. The ultimate goal of this ongoing study is to reconstruct phylogenetic relationships for all sections and subgroups within *Solanum* to achieve a detailed picture of evolutionary patterns in *Solanum* and its relatives.

Materials and Methods

Eighteen species of *Solanum*, representing 15 sections and five of the seven subgenera of D'Arcy (1972, 1991) were sequenced for *ndhF*. Species from the genera *Capsicum*, *Datura* L., *Jaltomata*, *Lycianthes*, and *Physalis* L. from subfamily Solanoideae also were sampled. *Nicotiana tabacum* L. from subfamily Cestroideae (sensu D'Arcy 1979 and Hunziker 1979) was included as an outgroup. Taxa were chosen to represent a broad spectrum of the diversity present in *Solanum*. Where possible, sampled species were identical to or parallel with those used in Olmstead and Palmer (1997). Several species were chosen to examine particular taxonomic problems [e.g., the placement and relationships of *S. allophyllum* (Miers) Standl., *S. wallacei* (A. Gray) Parish, and *S. wendlandii* Hook.]. Sampling and voucher data are given in Table 1.

DNA was extracted from fresh or silica-dried leaf samples by the modified CTAB method (Doyle and Doyle 1987). Extracts were purified by cesium chloride density gradient centrifugation. PCR amplification of the *ndhF* region was accomplished using primers 1 and 2110R of Olmstead and Sweere (1994) and the following PCR program: 92°C for 7 min, followed by 35 cycles of 92°C for 1 min, 45°C for 1 min, and 72°C for 5 min, with a single cycle of 72°C for 7 min. Primer 1 begins at position 1 of the tobacco coding sequence, and the end of primer 2110R closest to the 5′ end of the gene corresponds to position 2110 in tobacco (Olmstead and Sweere 1994). One primer in each PCR reaction was biotin-labeled, and purification of the double-stranded PCR products and generation of single-stranded DNA followed a streptavidin bead protocol (Dynal, Inc., Lake Success, NY). Manual sequencing was carried out with the Sequenase version 2.0 kit (United States Biochemical, Cleveland, OH) using the internal sequencing primers given in Olmstead and Sweere (1994), except that a new primer, 163F (5′-CAATCTACCTGTCTATTCAGC-3′), was designed. Missing data totaled 0.02% of the cells in the data matrix. All new sequences obtained in this study have been submitted to GenBank. The complete data set and trees depicted in Figs. 1–4 have been submitted to TreeBASE.

Sequences were aligned by eye and analyzed by parsimony and maximum likelihood methods. Maximum likelihood methods are considered especially useful in overcoming long branch attraction problems that may adversely affect the results of

TABLE 1. Sources of DNA accessions sequenced for *ndhF*. [a]DNA extracts provided by: 1—L. Bohs, University of Utah, Salt Lake City, UT. 2—R. G. Olmstead, University of Washington, Seattle, WA. 3—T. Mione, Central Connecticut State University, New Britain, CT. [b]Collector and number of herbarium vouchers. Bohs vouchers are at UT, RGO vouchers at WTU. BIRM samples bear the seed accession number of the University of Birmingham Solanaceae collection. [c]Same DNA accession used in Olmstead and Palmer (1992, 1997). [d]Corrected sequence from Olmstead et al. (1993). [e]As "*S. americanum*" in Olmstead and Palmer (1992). [f]Collection number from Sturgeon Bay USDA station. Sample also bears the annotation "PI (245793 × 245796)." [g]As gradum ambiguum in Dunal (1852). [h]Subgeneric assignment debated (see text).

Taxon	Subgenus	Section	Source[a]	Voucher[b]	GenBank accession numbers
Capsicum baccatum L. var. *pendulum* (Willd.) Eshbaugh			2	*Eshbaugh 1584*[c]	U08916
Datura stramonium L.			2	*RGO S-16*[c]	U08917
Jaltomata procumbens (Cav.) J. L. Gentry			3	*Davis 1189A*	U47429
Lycianthes heteroclita (Sendtn.) Bitter			1	*Bohs 2376*	U72756
Lycianthes lycioides (L.) Hassl.			2	*RGO S-87*	U73797
Nicotiana tabacum L.			2	none[c,d]	L14953
Physalis alkekengi L.			2	*D'Arcy 17707*[c]	U08927
Solanum abutiloides (Griseb.) Bitter & Lillo	*Minon*	*Brevantherum*	2	BIRM S. 0655	U47415
Solanum allophyllum (Miers) Standl.	Unassigned	*Allophyllum*	1	*Bohs 2339*[c]	U47416
Solanum arboreum Dunal	*Solanum*	*Geminata*	1	*Bohs 2521*	U47417
Solanum aviculare G. Forst.	*Archaesolanum*	*Archaesolanum*	2	BIRM S. 0809[c]	U47418
Solanum betaceum Sendtn.	Unassigned	Unassigned	1	*Bohs 2468*[c]	U47428
Solanum dulcamara L.	*Potatoe*	*Dulcamara*	2	none[c]	U47419
Solanum laciniatum Aiton	*Archaesolanum*	*Archaesolanum*	1	*Bohs 2528*	U47420
Solanum luteoalbum Pers.	Unassigned	*Cyphomandropsis*	1	*Bohs 2337*[c]	U72749
Solanum lycopersicum L.	*Potatoe*	*Lycopersicum*	2	none[c]	U08921
Solanum physalifolium Rusby var. *nitidibaccatum* (Bitter) Edmonds	*Solanum*	*Solanum*	1	*Bohs 2467*	U47421
Solanum pseudocapsicum L.	*Minon*	*Pseudocapsicum*	2	BIRM S. 0870[c]	U47422
Solanum ptychanthum Dunal	*Solanum*	*Solanum*	2	*RGO S-94*[c,e]	U47423
Solanum rostratum Dunal	*Leptostemonum*	*Androceras*	1	none	U47424
Solanum seaforthianum Andrews	*Potatoe*	*Jasminosolanum*	2	BIRM S. 0051	U47425
Solanum torvum Swartz	*Leptostemonum*	*Torva*	2	BIRM S. 0839[c]	L76286
Solanum tuberosum L. ssp. *tuberosum*	*Potatoe*	*Petota*	2	WRF 1610[c,f]	L76287
Solanum wallacei (A. Gray) Parish	*Solanum*	*Subdulcamara*[g]	1	*Bohs 2438*	U47426
Solanum wendlandii Hook.	*Leptostemonum*[h]	*Aculeigerum*	2	BIRM S. 0488	U47427

parsimony algorithms (Felsenstein 1978, 1981). Parsimony and maximum likelihood analyses were conducted using a test version 4.0d49 of PAUP provided by D. L. Swofford (Laboratory of Molecular Systematics, Smithsonian Institution, Washington, D.C.). In the parsimony analyses, the heuristic search algorithm was utilized with the TBR and MULPARS options and 100 random-order entry replicates. Bootstrap analysis was performed with 500 replicates using the heuristic search option with TBR branch swapping and MULPARS. Parsimony analyses were performed 1) with all nucleotide changes weighted equally; 2) with transition: transversion (ts/tv) ratios of 1.5 and 2; 3) with weights of 1.2:1:2.9 for first, second, and third position codons, respectively, and 4) with both ts/tv ratios and codon weights. For maximum likelihood, ten random-order entry replicate analyses were performed with all changes equiprobable and with ts/tv probability ratios of 1.5 and 2. Ten replicate analyses were performed with probability ratios of 1.2:1:2.9 for first, second, and third position nucleotide changes with ts/tv probability ratios of 1, 1.5, and 2. The probability values for change at codon positions correspond to the empirical number of differences at each position observed in the data set determined by pairwise sequence comparisons.

RESULTS

Two thousand eighty six nucleotides of DNA sequence were obtained for each taxon, corresponding to positions 24 through 2,109 in the tobacco *ndhF* sequence. The only exceptions were *Solanum wendlandii*, which had a 33 bp insertion at position

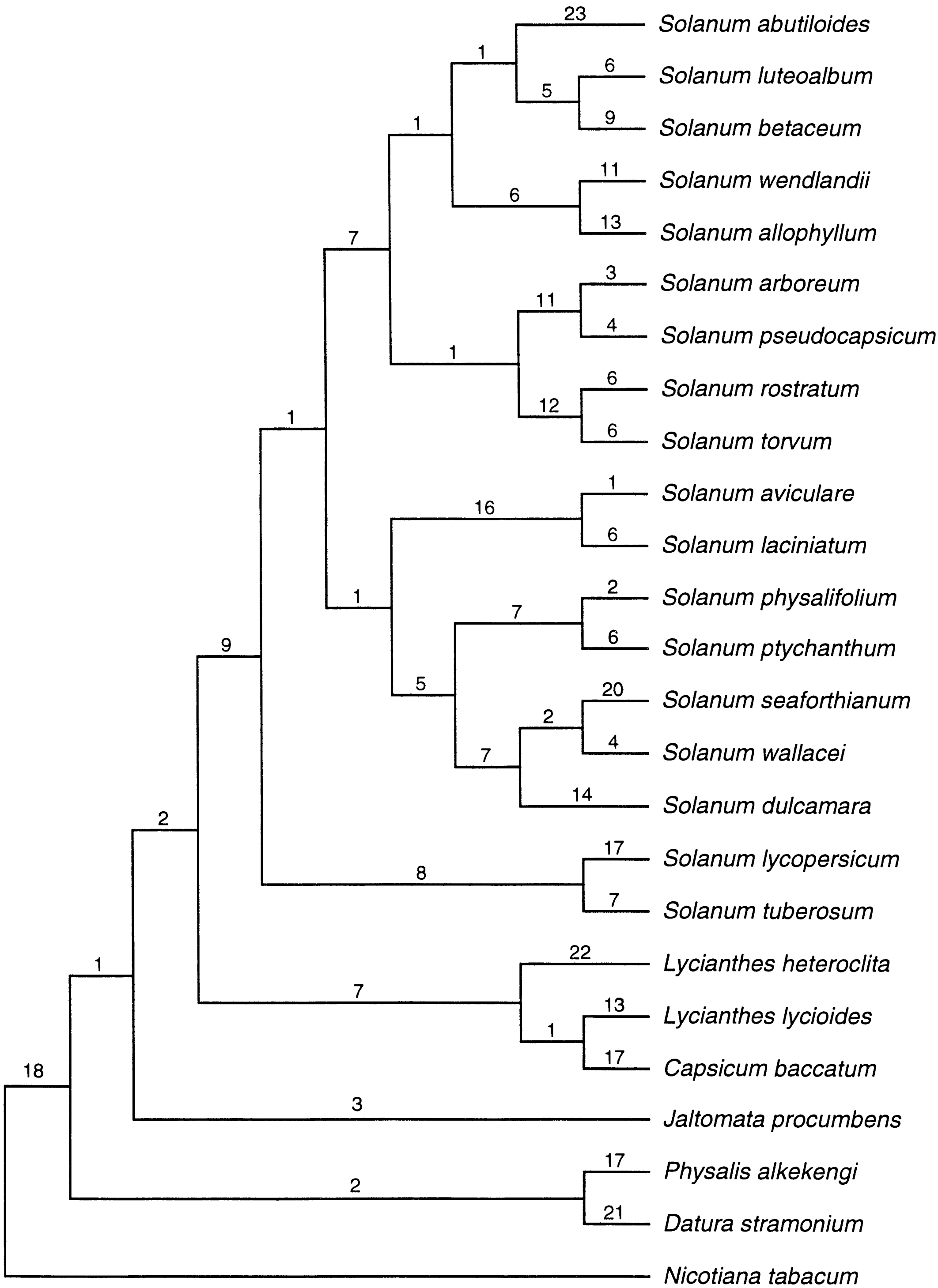

FIG. 1. One of 12 most parsimonious trees of 382 steps (CI = 0.682 excluding uninformative characters, RI = 0.795) from the unweighted parsimony analysis. Numbers represent nucleotide changes supporting each branch.

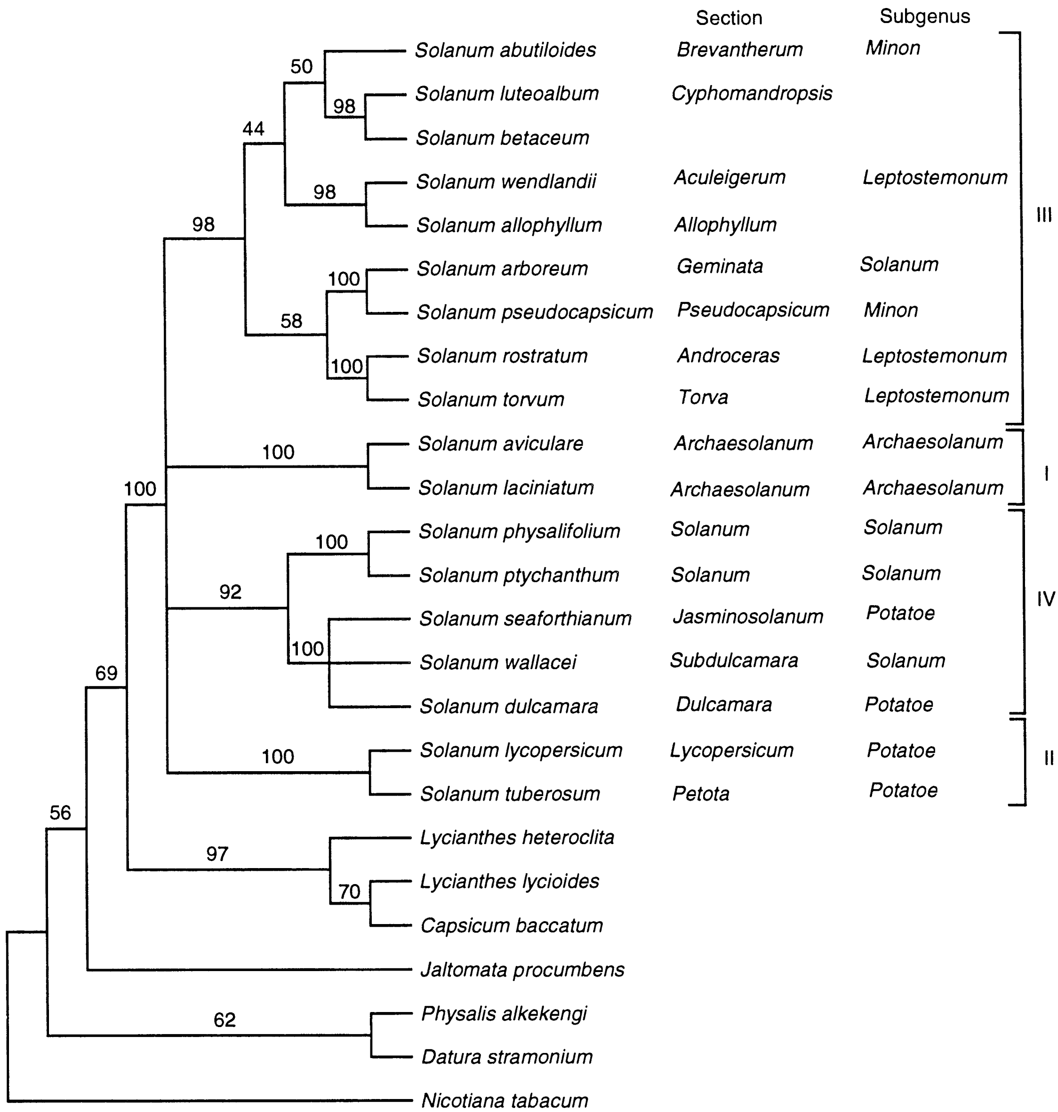

FIG. 2. Strict consensus tree derived from the 12 most parsimonious trees from the unweighted parsimony analysis. Numbers indicate percentage of bootstrap replicates supporting each clade (500 total replicates). Roman numerals and brackets indicate primary clades recognized in this analysis. Subgeneric and sectional classification follows D'Arcy (1972, 1991). *Solanum luteoalbum*, *S. betaceum*, and *S. allophyllum* have not been assigned to a *Solanum* subgenus, and *S. betaceum* has not been assigned to a section.

1,474, and *Lycianthes heteroclita* (Sendtn.) Bitter, which had a 15 bp insertion at position 1477. Both of these length variants were excluded from the analysis. The first 23 and last 26 bp of the coding sequence amplified for *ndhF* corresponded to the amplification primers, and were removed before analysis. All sequences were easily alignable by eye. Sequence divergence, calculated by direct pairwise comparisons uncorrected for multiple substitutions, ranged from 3.2% to 0.3%. The data set contained 301 variable characters, of which 115 were phylogenetically informative. The ts/tv ratio, as estimated from unambiguous changes inferred over one of the shortest trees (Fig. 1), was 1.1.

The lower boundary of phylogenetic utility of *ndhF* sequence data was examined by including two closely related species pairs, *S. physalifolium* Rusby and *S. ptychanthum* Dunal of section *Sola-*

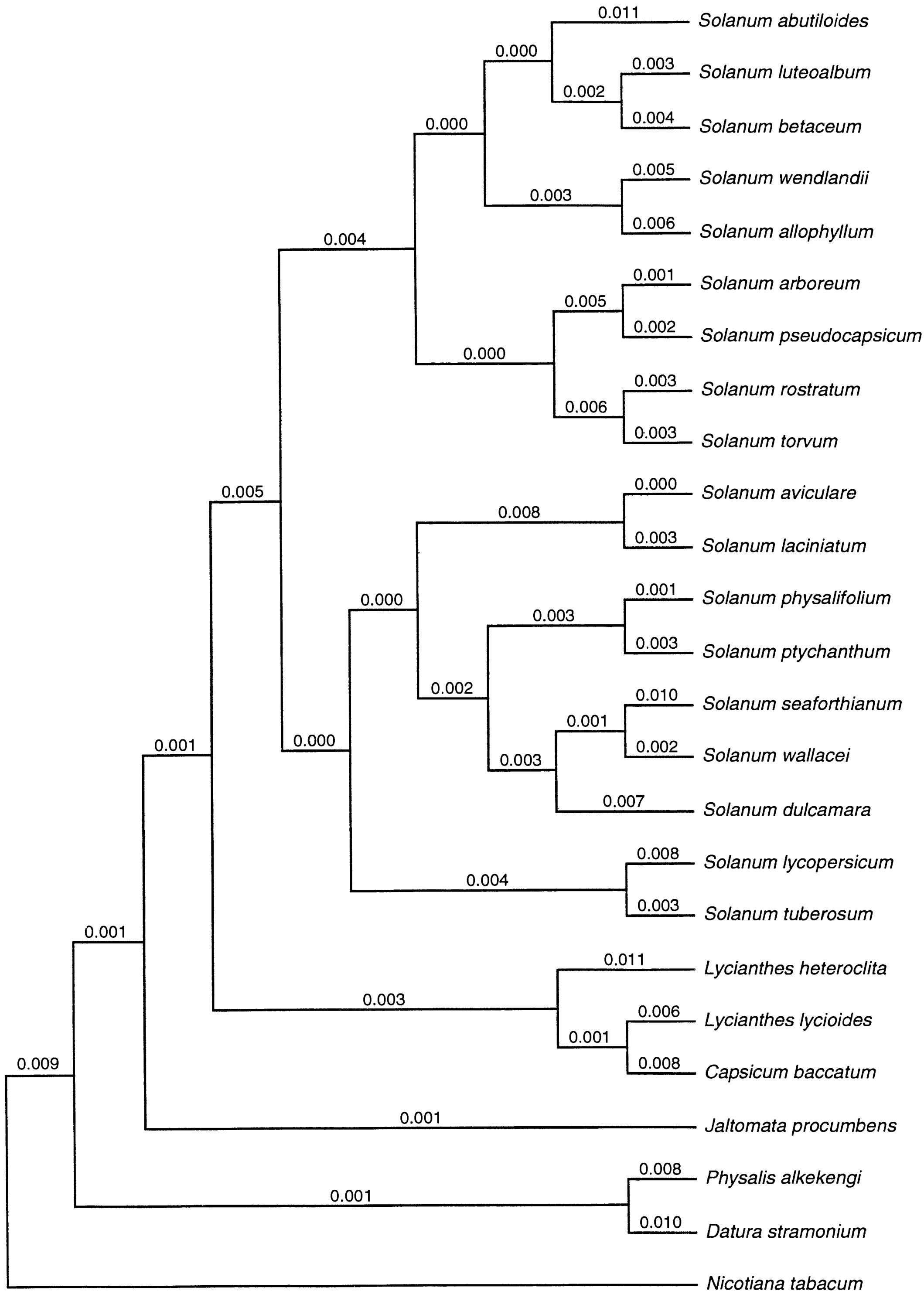

FIG. 3. Single tree topology obtained from ten replicates of the maximum likelihood analysis with equiprobable transformation rates. The same tree topology was obtained in maximum likelihood analyses using three categories of base substitution probability corresponding to codon position and transition:transversion probability ratios of 1, 1.5, and 2. Branch lengths are expected nucleotide substitutions per site.

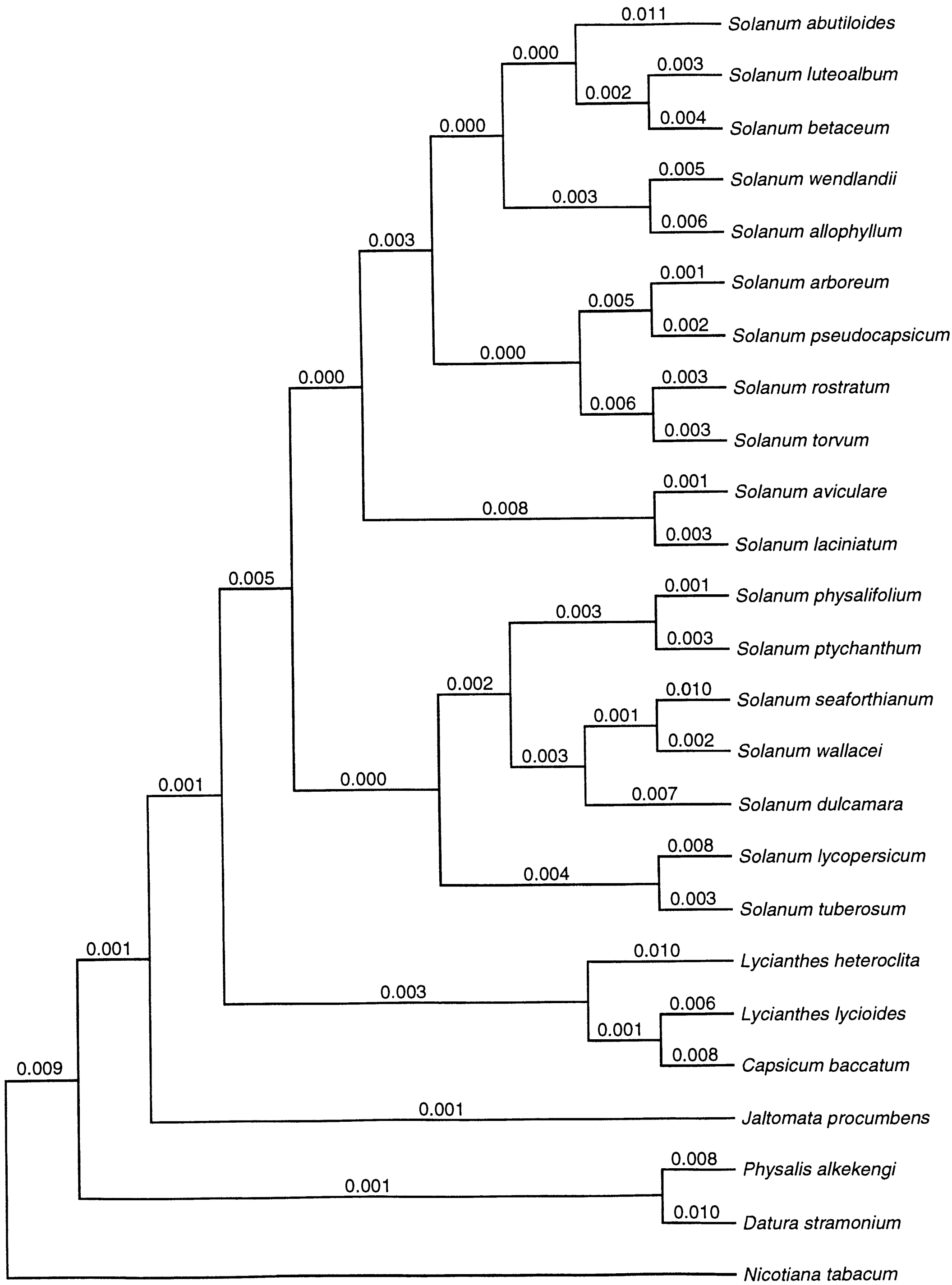

FIG. 4. Single tree obtained from ten replicates of the maximum likelihood analysis using transition:transversion probabilities of 1.5 and 2 with equal probabilities of change at codon positions. Branch lengths are expected nucleotide substitutions per site.

num, and *S. aviculare* G. Forst. and *S. laciniatum* Aiton of the small subgenus *Archaesolanum*. In all analyses, the species of the pairs formed well-supported clades. Mean sequence divergence was 0.4% between *S. physalifolium* and *S. ptychanthum* and 0.3% between *S. aviculare* and *S. laciniatum*, indicating that *ndhF* sequence data probably will have limited value for phylogenetic inference at taxonomic ranks below the level of section in *Solanum*.

The 100 replicate searches in the parsimony analysis using equal weights for all nucleotide positions resulted in 12 most parsimonious trees of 382 steps, with a consistency index (CI) of 0.835 (0.682 excluding uninformative characters) and retention index (RI) of 0.795 (Fig. 1). The strict consensus tree of these 12 most parsimonious trees is well-resolved, with polytomies occurring only at the base of the *Solanum* clade and in the clade composed of *S. dulcamara* L., *S. seaforthianum* Andrews, and *S. wallacei* (Fig. 2). The bootstrap analysis (Fig. 2) revealed strong support for the clade that includes all *Solanum* species. Four major clades can be recognized within *Solanum*: 1) *S. aviculare* and *S. laciniatum* (clade I on Fig. 2); 2) tomato (*S. lycopersicum* L.) plus potato (*S. tuberosum* L.) (clade II); 3) a morphologically diverse group represented by *S. arboreum* Dunal, *S. pseudocapsicum* L., *S. rostratum* Dunal, *S. torvum* Swartz, *S. wendlandii*, *S. allophyllum*, *S. abutiloides* (Griseb.) Bitter & Lillo, *S. luteoalbum* Pers., and *S. betaceum* Sendtn. (clade III), and 4) *S. seaforthianum*, *S. wallacei*, *S. dulcamara*, *S. physalifolium*, and *S. ptychanthum* (clade IV). Clades II and III are congruent with clades II and III, respectively, in Olmstead and Palmer (1997), while clades I and IV together comprise clade I in Olmstead and Palmer (1997). Within these major clades, several strongly supported groups of two or three species can be identified (Fig. 2).

All parsimony analyses using codon weighting resulted in three most parsimonious trees, all of which are a subset of trees found in the analysis using equal weights. The strict consensus of the codon-weighted trees differed from that of the equally-weighted trees in that clades I and III were sister taxa, and the relationships among *S. seaforthianum*, *S. wallacei*, and *S. dulcamara* were fully resolved, with *S. seaforthianum* basal in the clade and *S. wallacei* and *S. dulcamara* as derived sister taxa. The parsimony analysis conducted using a 1.5 ts/tv ratio also resulted in three most parsimonious trees which differed from those in the codon-weighted analyses only in the resolution of the three taxa mentioned above; in these trees, *S. seaforthianum* and *S. wallacei* were sister taxa, with *S. dulcamara* basal in the clade. The parsimony analysis using a ts/tv ratio of 2 recovered six most parsimonious trees, three of which were identical to the trees from the 1.5 ts/tv weighting. The other three trees differed only in collapsing the *Physalis* plus *Datura* clade.

The maximum likelihood analyses using ts/tv probability ratios of 1 and those using a combination of three categories of base substitution probability corresponding to codon position with ts/tv probability ratios of 1, 1.5 and 2 resulted in the same tree topology (Fig. 3). One tree topology also was found in the analyses using ts/tv probabilities of 1.5 and 2 with equal probabilities of change at codon positions (Fig. 4). The two trees did not differ significantly from each other according to the tree comparison test of Kishino and Hasegawa (1989). Both of these topologies were included in the set of 12 most parsimonious trees found in the parsimony analysis using equal weights.

The trees produced by both algorithms were largely congruent. Altering probabilities by codon position and changing the ts/tv probability ratio had little effect on tree topologies, and changes were confined to areas of the trees that were poorly supported in either analysis. The areas of conflict among the trees in all analyses were confined to resolution within the *S. seaforthianum/wallacei/dulcamara* clade and the *Physalis/Datura* clade, and to the relative positions of *Solanum* clades I (subgenus *Archaesolanum*) and II (subgenus *Potatoe*, pro parte).

All analyses resulted in the following conclusions: 1) the genera *Lycopersicon* (as *S. lycopersicum*) and *Cyphomandra* (as *S. betaceum*) are nested within *Solanum*; 2) if *Lycopersicon* and *Cyphomandra* are included, *Solanum* is well-supported as a monophyletic group; 3) four clades can be distinguished within *Solanum*, and 4) these clades are not necessarily congruent with the currently recognized subgenera of *Solanum*.

Discussion

The position of the genera *Lycopersicon* and *Cyphomandra* with respect to *Solanum* has been debated intensely and has been resolved only recently. This study, as well as those of Olmstead and Palmer (1992, 1997), Spooner et al. (1993), Olmstead et al. (in press), and Bohs and Olmstead (in press) have used cpDNA restriction site and sequence data to establish that these two genera are nested within the *Solanum* clade. The species of both genera have been transferred to *Solanum* (Spooner et al. 1993; Bohs 1995), rendering *Solanum* monophyletic, at least as far as the sampled taxa are concerned.

The results indicate that four of the five currently accepted subgenera of *Solanum* sampled in this study probably are not monophyletic. The exception is the small (ca. 10 species) subgenus *Archaesolanum*, a morphologically distinctive group with a base chromosome number of $n = 23$ whose species are confined to Australia, New Zealand, and New Guinea (Symon 1994). *ndhF* sequence data were obtained for two members of subgenus *Archaesolanum*, and they emerge as sister taxa in all analyses and have 100% bootstrap support. However, the position of subgenus *Archaesolanum* in relation to other lineages in *Solanum* is still unclear. *Archaesolanum* is joined to a clade composed of *S. seaforthianum*, *S. wallacei*, *S. dulcamara*, *S. physalifolium*, and *S. ptychanthum* in a subset of the parsimony trees and one of the maximum likelihood trees, but with little support. An alternative placement, as basal to *Solanum* clade III, is also suggested by some of the analyses. The only other molecular phylogenetic study that includes a representative of subgenus *Archaesolanum* is that of Olmstead and Palmer (1997) based on cpDNA restriction site data. The placement of subgenus *Archaesolanum* in their study, basal in a clade composed of members of sections *Solanum*, *Dulcamara* Dumort., and *Jasminosolanum* Seithe, is congruent with some of the *ndhF* results. Symon (1994) surmises that the closest relatives of subgenus *Archaesolanum* are to be found within subgenus *Solanum*, but he does not narrow the possibilities further. The *ndhF* data and those of Olmstead and Palmer (1997) both suggest that the ancestor of the *Archaesolanum* clade arrived in Australasia early in the evolutionary radiation of *Solanum*. Further taxonomic sampling or examination of additional data may be needed to resolve the position of subgenus *Archaesolanum* within the larger context of the genus.

Three species (*S. rostratum*, *S. torvum*, and *S. wendlandii*) were sampled from subgenus *Leptostemonum* s.l. The former two species are typical members of the subgenus; they are prickly plants with stellate hairs. *Solanum wendlandii* is anomalous in subgenus *Leptostemonum* because it has prickles but lacks stellate hairs. Although most modern workers (e.g., D'Arcy 1972, 1991; Whalen 1984) have included *S. wendlandii* in subgenus *Leptostemonum*, others have removed it from the subgenus and placed it either into subgenus *Solanum* (Seithe 1962) or subgenus *Potatoe* (Child 1990). Danert (1970) does not place *S. wendlandii* and the other members of section *Aculeigerum* Seithe in a subgenus, but he considers the group to be closely related to section *Jasminosolanum*. Results from the *ndhF* study indicate that *S. rostratum* and *S. torvum* form a monophyletic group, but that *S. wendlandii* lies outside this clade and is not sister to it. Furthermore, *S. wendlandii* does not group with members of subgenus *Solanum*, subgenus *Potatoe*, or section *Jasminosolanum*, but instead is strongly supported as sister to *S. allophyllum*. Prickles apparently have arisen independently in *S. wendlandii* and in subgenus *Leptostemonum*. Subgenus *Leptostemonum* may be monophyletic if *S. wendlandii* and its relatives are excluded.

Solanum allophyllum and its relatives represent another enigmatic group in *Solanum*. *Solanum allophyllum* and two other species comprise the section *Allophyllum* (Child) Bohs (Bohs 1990), but the section has not yet been placed in a subgenus. The members of section *Allophyllum* lack spines and stellate hairs, but have tapered anthers that resemble those of subgenus *Leptostemonum* and the section *Aculeigerum* to which *S. wendlandii* belongs. According to the *ndhF* data, *S. allophyllum* and *S. wendlandii* are sister taxa, indicating a close relationship of sections *Allophyllum* and *Aculeigerum*. Moreover, this clade is not sister to subgenus *Leptostemonum*, indicating that tapered anthers may have evolved more than once in *Solanum*.

Subgenus *Minon* Raf. [subgenus *Brevantherum* (Seithe) D'Arcy sensu D'Arcy (1972)] also apparently is not monophyletic as currently circumscribed. Two representatives of the subgenus were included in the *ndhF* study (*S. pseudocapsicum* and *S. abutiloides*), and they did not emerge as sister taxa. Instead, *S. pseudocapsicum* is strongly supported as sister to *S. arboreum* of section *Geminata*. Olmstead and Palmer (1997), using cpDNA restriction site data, also come to this conclusion, although they sampled a different representative from section *Geminata*, *S. aphyodendron* Knapp. D'Arcy (1972, 1991) places section *Geminata* in subgenus *Solanum* and section *Pseudocapsicum* Bitter in subgenus *Minon*, although S. Knapp (pers. comm.) considers section *Pseudocapsicum* to be closely related to or even included in section *Geminata*. The *ndhF* and restriction site results support the latter view and argue for removal of section *Geminata* from subgenus *Solanum* and placement in subgenus *Minon*. *Solanum abutiloides*, the other presumed member of subgenus *Minon* included in the study, is well removed from the *S. pseudocapsicum*/*S. arboreum* clade. Morphologically, sections *Pseudocapsicum* and *Brevantherum* have little in common except for the presence of short, blunt anthers and white corollas, both of which may be plesiomorphic character states in *Solanum* (Bohs, unpubl. data). Because subgenus *Minon* is typified by *S. pseudocapsicum*, *S. abutiloides* and other members of section *Brevantherum* must be placed in a different subgenus. The subgeneric name *Brevantherum* (Seithe) D'Arcy is available and may be used for this purpose if additional phylogenetic studies point to recognition of section *Brevantherum* at the subgeneric rank.

Solanum luteoalbum, a member of section *Cyphomandropsis* Bitter, is strongly supported as sister to *S. betaceum*. This is in accordance with morphological and cytological data, which ally the section with members of the former genus *Cyphomandra* (Bitter 1913; D'Arcy 1972; Child 1984; Pringle and Murray 1991; Moscone 1992; Bohs 1994). Chloroplast DNA restriction site data also indicate that the two groups are closely related (Olmstead and Palmer 1992, 1997). Furthermore, the *ndhF* data suggest that *S. betaceum* and *S. luteoalbum* may be part of a clade that includes *S. abutiloides* of section *Brevantherum*. This relationship has not been suggested by previous workers, and the clade is not strongly supported (Figs. 1, 2). Further sampling of genes or taxa will be needed to resolve relationships within *Solanum* clade III.

Subgenus *Solanum* remains polyphyletic even if *S. arboreum* and the remainder of section *Geminata* are removed from it. Species included in the *ndhF* analyses that have been included in subgenus *Solanum* are *S. wallacei*, *S. physalifolium*, and *S. ptychanthum*. *Solanum physalifolium* and *S. ptychanthum* come out as sister taxa, consistent with their many morphological similarities and traditional placement together in section *Solanum*. *Solanum wallacei* is nested within a strongly supported clade that includes *S. seaforthianum* and *S. dulcamara*. The latter two species are placed in subgenus *Potatoe* in D'Arcy's (1972, 1991) schemes. Other members of subgenus *Potatoe* sampled include *S. tuberosum* and *S. lycopersicum*, which come out together in a well-supported clade. The *ndhF* results agree with those of Olmstead and Palmer (1997) and indicate that both subgenera *Solanum* and *Potatoe* are not monophyletic as currently circumscribed. Future work may show that taxa such as *S. seaforthianum* and *S. dulcamara* should be transferred to subgenus *Solanum* and that subgenus *Potatoe* should be more narrowly defined to include only potatoes, tomatoes, and their close relatives. However, Spooner et al. (1993) found a different placement for *S. nigrum* L. of subgenus *Solanum* that argues for inclusion of sections *Dulcamara* and *Jasminosolanum* in subgenus *Potatoe*. A definitive resolution of this problem must await additional sampling in other groups of the non-spiny solanums.

This study, like other molecular studies (Olmstead and Palmer 1992, 1997; Spooner et al. 1993; Bohs and Olmstead, in press; Olmstead et al., in press) refutes the idea of a fundamental division of *Solanum* into two large groups based either on anther or trichome characters. For instance, Dunal (1852) divided the genus into two "sections" (the ranks of many of Dunal's infrageneric categories are ambiguous; [see, for example, D'Arcy (1972) and Knapp (1983)], *Pachystemonum* Dunal and *Leptostemonum* Dunal, based on anther shape (short and broad vs. long and tapered toward the apex). Even if subgenus *Leptostemonum* is redefined to make it monophyletic by removal of section *Aculeigerum*, the remainder of *Solanum* species forms a para- or polyphyletic assemblage that cannot be recognized as a taxonomic unit in a phylogenetic classification scheme (deQueiroz and Gauthier 1992). Seithe (1962) also divided the genus into two large groups, referred to as "chorus subgenera" at a rank between genus and subgenus, using hair morphology as the primary criterion for assigning taxa. Chorus Subgenerum *Solanum* (L.) Seithe included species with unbranched or dendritically branched hairs, whereas Chorus Subgenerum *Stellatipilum* Seithe encompassed taxa with stellate hairs. Among the species sampled for *ndhF*, *S. rostratum*, *S. torvum*, and *S. abutiloides* possess stellate hairs, and these taxa do not form a monophyletic group.

Both the *ndhF* data presented here and the cpDNA restriction site data of Olmstead and Palmer (1997) indicate that *Lycianthes* and *Capsicum* form a clade, with *Capsicum* derived from within *Lycianthes*. Bitter (1920) previously suggested a close relationship between *Capsicum* and *Lycianthes*, but he included in the alliance genera such as *Witheringia* L'Her., which is shown to belong with the physaloid genera in the molecular analyses of Olmstead et al. (in press). However, the results of Olmstead and Palmer (1997) differ from those of the *ndhF* study in placing *Jaltomata* rather than the

Lycianthes/Capsicum clade as sister to *Solanum*. *Capsicum* was sister to *Solanum* in the earlier analysis of *ndhF* data by Bohs and Olmstead (in press), but the bootstrap value for the clade was low (64%) and *Lycianthes* was not sampled. Olmstead et al. (in press) found that *Capsicum* emerged as the sister group to *Solanum* in some analyses based on combined restriction site and sequence data, but that *Jaltomata* resulted as the sister group when more taxa were included. The restriction site data of Olmstead and Palmer (1992, 1997) also argue for *Jaltomata* rather than *Capsicum* as sister to *Solanum*, although the bootstrap values for the clade (48–61%) are not indicative of strong support. Additional data for other representatives of the tribe are needed to pinpoint the sister group to *Solanum*.

There is good congruence between the trees derived from *ndhF* sequence data and those from the cpDNA restriction site data of Olmstead and Palmer (1997). Although sampling differs somewhat in the two studies, the same major infrageneric groups are found. The consistent association of the species pairs *S. physalifolium/S. ptychanthum* and *S. aviculare/S. laciniatum* in the *ndhF* trees agrees with morphological data and the traditional placement of the species in the same sections. Congruence among trees derived from different cpDNA data sets is not surprising in light of the fact that cpDNA belongs to a single linkage group (Doyle 1992). A complete picture of the evolutionary history of this group of taxa must await comparison of results from phylogenetic studies using nuclear genes and/or morphological characters.

This study illustrates the potential of *ndhF* sequence data to reconstruct phylogenetic relationships among species of *Solanum* and related genera. Only a small fraction of the taxonomic diversity of *Solanum* was sampled, yet the results are intriguing and support additional sequencing efforts. Some of the major problems that remain to be solved are the phylogenetic relationships among genera of the tribe Solaneae, the patterns of evolution of deep lineages within *Solanum*, the placement of many problematical infrageneric taxa, comparison of trees based on molecular data with those derived from morphological characters, and examination of rates of evolution and speciation in *Solanum* and related taxa. Ultimately, data from *ndhF* and other genes combined with morphological, cytological, and biochemical characters may provide the most comprehensive view of evolutionary relationships in this important, widespread, and species-rich genus.

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